EFFECT OF SOIL MOISTURE ON DEVELOPMENT OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

We conducted trials to determine conditions of soil moisture required to optimize production of adults of *Diaprepes abbreviatus* (L.) in a laboratory colony. Larvae of *D. abbreviatus* were reared on a commercially available artificial diet and then placed in soil with water content ranging from 20 to 80%. Optimal moisture content of soil for pupation was determined to be 60 ± 10% by weight. When 68-d-old larvae were transferred from artificial diet to soil with these moisture levels and constant temperature (25°C), mean (± SEM) development time of *D. abbreviatus* from neonate to pupa was 126 ± 2.3 d (n = 47). For all pupae, the time required for pupation did not vary with soil moisture. When 68-d-old larvae were taken from diet and placed in soil, the proportion that pupated varied with moisture content. Low (20-40%) and high (80%) moisture content resulted in increased mortality, and fewest larvae pupated within the low range. The mean ± SEM number of days to pupation of 68-d-old larvae was 58.2 ± 2.3 (n = 47). Older (180 d) larvae reared on diet pupated over a greater range of moisture treatments (30-70%) and were adversely affected only by the highest (80%) and lowest (20%) treatments. Mean ± SEM time to pupation (38.4 ± 1.9 d, n = 54) did not vary for 180-d-old larvae kept at 30-70% moisture content.

Key Words: Development, soil moisture, pupation, citrus root weevil

RESUMEN

Realizamos ensayos para determinar las condiciones de humedad de suelo requeridas para optimizar la producción de adultos de *Diaprepes abbreviatus* (L.) en una colonia de laboratorio. Para determinar las condiciones óptimas de humedad para la pupación, se criaron larvas de *D. abbreviatus* con una dieta artificial comercial, y se colocaron en suelo con un contenido de agua entre 20 y 80%. El contenido óptimo de humedad del suelo fue de 60 ± 10% por peso. Cuando las larvas de 68 días de edad fueron transferidas de la dieta artificial al suelo en este rango de humedad y con temperatura constante de 25°C, el promedio (± error estandard del promedio) del tiempo de desarrollo de *D. abbreviatus* criada desde neonata hasta pupa fue de 126 ± 2.3 d (n = 47). Para las pupas, el tiempo requerido para la pupación no varió con diferentes tratamientos de humedad del suelo. De las larvas transferidas a los 68 d de dieta a suelo, la proporción que empupó varió con la humedad del suelo. Bajo (20-40%) y alto (80%) contenido de humedad causaron un incremento de mortandad; menos larvas empuparon en los tratamientos de baja humedad. El promedio del número de días hasta la pupación de larvas de 68 d fue de 58.2 ± 2.3 (n = 47). Las larvas de 180 d criadas con una dieta artificial empuparon bajo condiciones más amplias de humedad (de 30 a 70%) y su pupación fue menos solamente en el tratamiento más húmedo (80%) y más seco (20%). El promedio del tiempo a empupación (38.4 ± 1.9 d, n = 54) no varió para larvas de 180 d mantenidas en los tratamientos de 30 a 70% de humedad del suelo.

The root weevil *Diaprepes abbreviatus* (L.), is increasing in importance as a pest of Florida citrus and ornamentals as its range expands throughout central and southern portions of the state. Twenty counties currently are listed as infested by the Florida Department of Agriculture and Consumer Services. This weevil is remarkable for its wide host range and the severity of damage inflicted on individual citrus trees (Simpson et al. 1996, Schroeder & Sutton 1977). Timely detection of larvae and adults of *D. abbreviatus* is difficult and few control options are available. A colony of *D. abbreviatus* was established at the U.S. Horticultural Research Laboratory at Orlando, FL in 1992. The rearing method has developed empirically and little is understood about the effect of temperature and soil moisture content on larval developmental rate and pupation. Developmental time under current colony conditions is highly variable making rearing costly and inefficient. Under colony conditions, pupation is irregular and occurs between 3 and 18 months after egg eclosion.

Beavers (1982) reported successful rearing of *D. abbreviatus* on an artificial diet and estimated the total development time to be approximately 1 year, as did Wolcott (1934). Wolcott felt pupation of *D. abbreviatus* occurred in the spring, presumably with the onset of rains based on observations of Barrow (1924). His observations that eggs occur in the field throughout the year argue for a flexible developmental plan wherein developmental time can vary from 6 months to more than a year. Beavers & Selhime (1986) also suggested increased rainfall precedes peaks in adult numbers. Recently, speculation has focussed on the possibility of genetic variability in developmental rate and time to pupation. However, to assess genetic variation in developmental traits, it will first be necessary to establish uniform, optimal rearing conditions to eliminate environmental effects on development and pupation.

Identification of host plant resistance in citrus and near-citrus relatives relies on bioassays involving larval feeding (Shapiro & Gottwald 1995, Shapiro et al. 1997). Efforts to measure aspects of plant-insect interactions and to assess efficacy of control options for this increasingly important pest will benefit from a definition of the environmental parameters required for development and pupation. Tarrant & McCoy (1989) characterized the effect of temperature on 3 genera of root weevils that attack citrus, but did not include *D. abbreviatus*. A brief report by Schroeder (1987) alluded to the potential for inducing pupation in laboratory-reared *D. abbreviatus* by transferring larvae from diet to soil. We report here the results of a controlled study of the response of larval *D. abbreviatus* to conditions of soil moisture.

Materials and Methods

Rearing.

All stages of *D. abbreviatus* were reared at the U.S. Horticultural Research Laboratory, Orlando, FL. Eggs were collected from caged adults on wax-paper strips (Wolcott 1933) and allowed to eclose in plastic containers. Diet for larval development was prepared as follows: 40 liters of water were combined with 725 g agar and heated to near boiling. While stirring, 9.5 kg of commercially prepared insect diet [product no. F1675, Bio-Serv, Inc., Frenchtown, NJ, similar to that developed by Beavers (1982)] were added to the water/agar mixture, mixed, and heated to between 200 and 230°C. Methylparaben (9 g dissolved in 10 ml 95% EtOH) and 9 g of benzoic acid in solution with boiling deionized water were added as preservatives. After 10 min. of boiling, ~15 ml of diet was dispensed into 30-ml plastic cups and allowed to cool and dry in a laminar flow hood. Neonate larvae were surface sterilized for ~2 min. in a 0.25% bleach solution, rinsed with deionized water, and placed in cups with diet. The cups were covered with plastic lids (PC100 1 oz. cups and lids, Jet Plastica, Harrisburg, PA). Trays

containing the cups were held in a room at 25°C and 60-70% RH. Because of mortality associated with larval interactions in the cups, cups were opened at ~4-6 wk and individual larvae were transferred to fresh diet cups to complete development. Larvae pupated in the diet cups. Teneral adults were left for at least 3 d until sclerotization of the cuticle was complete. Adults were fed green beans, carrots, or citrus foliage according to availability and intended experimental design. Field-collected adults were introduced when available from the field to renew the colony and avoid adaptation to rearing conditions.

Because the objective of mass-rearing has been to provide insects for experimental purposes, progress has been empirical and output-oriented. We initiated a series of experiments to quantify the effect of environmental parameters on the developmental physiology of *D. abbreviatus*.

Trial 1.

Soil (Metromix 500, Scotts, Marysville, OH) was dried at 60°C for 4 d in an analytical oven and weighed. Water was added to dry soil to generate soil moisture levels of 20 to 80% in 5% increments. We placed 105- and 180-d-old larvae from the colony into cups containing soil without diet and kept them at room temperature (~25°C). Fifteen larvae (reps) were removed randomly from the colony without regard to their weight and assigned to each treatment. Ambient relative humidity in the rearing room where the cups containing the larvae were stored fluctuated around 70%. Because lids do not form a perfect seal, soil moisture content declined by as much as 25% over the course of the experiment (12 wk). Larvae were observed every 2 wk for pupation. Dates of pupation and adult emergence were recorded until the end of the trial (12 wk).

Trial 2.

A second trial was designed to maintain more uniform temperature and moisture conditions throughout the trial. Treatments were reduced to 7 (20-80% soil moisture content in 10% increments) and prepared as in Trial 1. We randomly selected 68- and 180-d-old larvae from the colony and placed them into cups containing soil without diet. The younger larvae (68 d) were selected to represent late instars close to completion of development. Older larvae (180 d) were selected because their pupation was delayed, possibly due to inadequate conditions in the colony. Each treatment (larval age × moisture) was sealed in a plastic bag to minimize moisture loss. Bags containing larvae and soil were kept in a dark incubator at 25 ± 1°C. Larvae were observed every 2 d for pupation. Dates of pupation and adult emergence were observed until the end of the trial (14 wk). Fresh and dry weights were determined at the end of the trial for 5 insects from each treatment. Insects were weighed upon removal from their cups, dried in an analytical oven at 60°C for 4 d and weighed again. Data were analyzed by analysis of variance and Tukey's Honestly Significant Differences (HSD) test (Abacus Concepts 1996).

Results and Discussion

Rearing.

Adults, larvae, and eggs of *D. abbreviatus* have been produced continuously since 1992 using the method described. Current peak production is approximately 400 adults/wk.

Trials 1 and 2.

Total pupation increased in Trial 1 with increasing soil moisture and reached a plateau at 60 - 65% for both age classes of weevils (Fig. 1). Ambient temperature and humidity were not controlled in this trial. We suspect that soil moisture content in the cups containing soil and larvae declined over the course of the experiment. In Trial 2, capped cups were kept in an incubator in sealed plastic bags at constant temperature, minimizing moisture loss. Optimal soil moisture for pupation of 68-d-old larvae ranged between 50 and 70% with a large increase in mortality at 80%. At 20% soil moisture content, mortality was also high and no pupation occurred (Fig. 2A). The time required for pupation did not vary by treatment ($\alpha = 0.05$, ANOVA) although sample size was small because few insects had pupated at the extreme treatments by the conclusion of the experiment at 90 d.

Mortality of older larvae (180 d) in Trial 2 was also high at 20 and 80% soil moisture content (Fig. 2B). Pupation was similar for treatments between 30 and 70%. Time to pupation was greater at 80% compared with the remaining treatments ($\alpha = 0.05$, Tukey's HSD test). Mean ± SEM time to pupation for the remaining treatments was 38.4 ± 1.9 d ($n = 54$).

Early experience with the colony indicated an apparent high degree of variability in time required for pupation, often exceeding one year (Beavers 1982). However, in Trial 2, of those 68-d-old larvae subjected to favorable moisture conditions (50-70%),

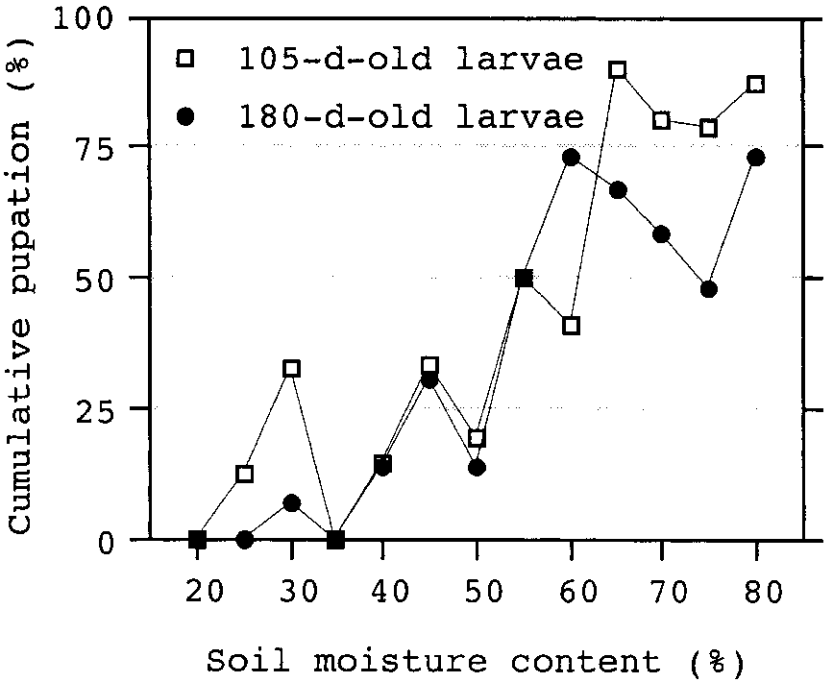

Fig. 1. Effect of initial soil moisture content on cumulative pupation of 105- and 180-d-old larval *D. Abbreviatus* after 12 wk.

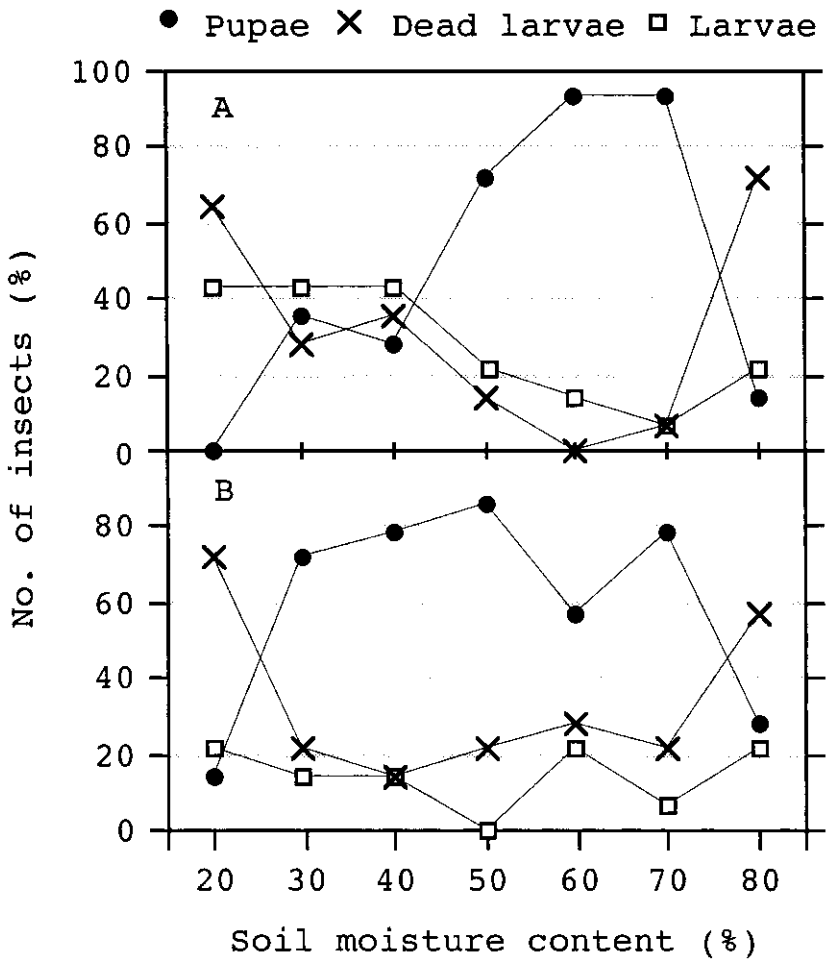

Fig. 2. Effect of constant soil moisture content on cumulative pupation and mortality of 68- (A) and 180-d-old (B) larval *D. abbreviatus*.

pupation occurred at 58 ± 3 d. The distribution of pupation times closely approached a normal distribution (Fig. 3). From these data, there does not appear to be genetic variability for rate of development.

There was no difference in the number of days to pupation of those 68-d-old larvae that pupated during Trial 2 (Table 1). The mean number of days (± SEM) to pupation of larvae transferred from diet to soil at 68 d was 58.2 ± 2.3 days after transfer (n = 47). Therefore, the mean development time of neonate larvae to pupation was 18 wk (~4.2 months). Fewer larvae survived at high (80%) and low (20-40%) soil moisture contents (Fig. 2A). Similarly, survival of 180-d-old larvae was low in the 20 and 80% treatments although in general, older larvae pupated over a greater range of moisture conditions compared with 68-d-old larvae (Fig. 2B). At 80% soil moisture content, the

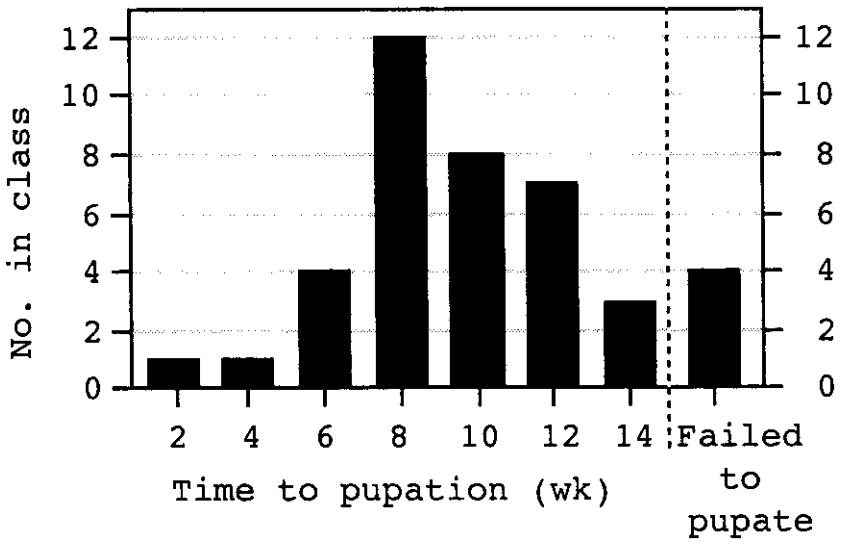

Fig. 3. Frequency distribution of time to pupation of 68-d-old larval *D. abbreviatus* in soil with moisture content of 50-70%.

development of 180-d-old larvae that pupated during the trial was significantly delayed compared with the remaining treatments (Table 1).

Soil moisture content did not affect the dry weights of insects at the end of Trial 2 (Pr > F = 0.12 and 0.14 for 68- and 180-d-old larvae, respectively). Final dry weights (mean ± SEM) were 122 ± 5 and 110 ± 6 mg for 68- and 180-d-old larvae, respectively. However, moisture content of insects at the end of the trial increased with increasing soil moisture content (Table 2). In the field, we expect that larvae of *D. abbreviatus* are capable of directed movements towards areas of preferred humidity in order to maintain their water content.

Barrow (1924) and Beavers & Selhime (1986) observed that heavy rainfall generally preceded emergence of adult Diaprepes in the field. Dry soil conditions may delay development resulting in an accumulation of dormant larvae or pupae in the soil. Subsequent rainfall may serve to synchronize completion of development, pupation, or adult emergence. Nothing is known about how larvae of *D. abbreviatus* move in the soil as a function of soil conditions (moisture content) and developmental stage. Wolcott (1934) observed that in arid and semi-arid regions of Puerto Rico, the weevil is present mostly in irrigated fields. It is possible that modern irrigation methods contribute to larval feeding damage by creating optimal conditions of soil moisture near the structural roots of citrus where the most severe feeding damage occurs.

Wolcott (1934) estimated the normal life cycle of *D. abbreviatus* to be approximately 1 year in the field based on observations of a small number of weevils reared in his laboratory in Puerto Rico. Beavers (1982) reported development times of slightly longer than 1 year for both sexes when reared on artificial diet. However, no attempt was made to control or monitor moisture conditions of the artificial diet. Our data suggest that Beavers' estimate of development time was largely an artifact of the rearing conditions, specifically, the progressive drying of the diet over time under laboratory conditions. Given optimal soil moisture (60 ± 10% by weight) and temperature condi-

TABLE 1. TIME REQUIRED FOR PUPATION AND EMERGENCE OF ADULTS AFTER TRANSFER OF 2 AGE CLASSES OF LARVAE OF *DIAPREPES ABBREVIATUS* TO SOIL WITH DIFFERENT WATER CONTENTS.

	68-d-old larvae						180-d-old larvae					
	Days to pupation			Teneral period (d)			Days to pupation			Teneral period (d)		
% water	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM	*n*
20	—		0	—		0	40.0 a	0.0	2	—		0
30	50.0 a	2.8	5	21.3 a	1.4	3	41.2 a	3.3	10	22.3 a	0.9	7
40	62.5 a	3.9	4	22.0 a	0.0	1	34.2 a	3.1	11	21.4 a	0.5	7
50	59.0 a	5.0	10	19.1 a	0.7	7	39.3 a	3.4	8	20.3 a	0.7	8
60	55.1 a	5.2	13	20.0 a	0.4	10	36.3 a	3.4	8	20.3 a	0.7	8
70	61.1 a	4.5	13	20.2 a	0.7	9	40.5 a	5.6	11	19.0 a	0.5	10
80	67.0 a	0.7	2	18.0 a	1.4	2	68.0 b	7.6	4	26.0 a	4.2	2

Means within columns followed by the same letter do not differ ($\alpha = 0.05$, ANOVA and Tukey's HSD).

TABLE 2. WATER CONTENT OF 2 AGE CLASSES OF *DIAPREPES ABBREVIATUS* AFTER 90 D ON SOIL WITH DIFFERENT SOIL MOISTURE CONTENT.

Soil water content (%)	Insect water content (%) (Mean ± SEM, n = 5)	
	68-d-old larvae	180-d-old larvae
20	44 ± 2 a	33 ± 8 a
30	53 ± 2 ab	33 ± 9 a
40	56 ± 2 b	43 ± 4 ab
50	60 ± 2 bc	60 ± 0 bc
60	67 ± 2 cd	67 ± 3 c
70	68 ± 2 cd	67 ± 2 c
80	72 ± 3 d	70 ± 2 c

Means within columns followed by the same letter do not differ (α = 0.05, Tukey's HSD).

tions (as yet to be determined), our estimate of the development time of *D. abbreviatus* reared on artificial diet and transferred to soil is approximately 18 wk at 25°C. It must be noted that we used larvae at moderate to advanced stages of growth (68 and 180 d) from the colony where moisture content of the diet and temperature were not controlled. Development time from neonate to adult may be even less if neonates are subjected to optimal conditions of moisture and temperature beginning at egg eclosion, and are provided with diet throughout the larval period. We are currently testing combinations of moisture and temperature to identify optimal rearing conditions.

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